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February 13, 2001

Mr. Erwin Sholts
Director
Agri Development & Diversification Program
State of Wisconsin
PO Box 8911
Madison, WI 53708-8911

Dear Mr. Sholts:

Please find enclosed a copy of the report we have prepared of the 1999 season study that was conducted on the use of a natural lipid lysophosphatidylethanolamine to enhance color and shelf life of Wisconsin cranberries. This project was partially funded by the Wisconsin State Agricultural Development and Diversification Program.

We want to thank you for providing these funds that made it possible to take this technology to the large scale commercial level.

As you will note, preharvest application of LPE to several beds, at Northlands Cranberries Inc, enhanced both the color and shelf life of cranberries fruits. This year we were able to enlarge the scope of these field trials and preliminary results are very encouraging. In fact, several of the treated fields growers could see a dramatic influence on the color.

Again, thank you for your support. We hope this all-natural lipid will become available to our growers in the near future and that this technology will allow our growers to increase profits.

Sincerely,

Dwight Triplett
President & CEO

Gregg Johnson
Vice President Business Operations

Report- results of LPE cranberry field trials
Fall 1999.

Experimental Design:

The experiment was conducted in three groups of plots:

1) Northland Cranberries Inc. Nakoosa (3 plots) , Cultivar Stevens

2) Ocean Spray Cranberries Inc. Habelman (4 plots), Cultivar Stevens

3) Ocean Spray Cranberries Inc. Habelman (4 plots), Cultivar Ben Lear

The **method of application** differed at the two properties. The Nakoosa plots received a closer to the ground, targeted application of LPE with a boom sprayer, avoiding of drift into unsprayed areas. The Habelmann plots received spray application with bridge mounted on two tractors.

The **surfactants used** at these two locations were also different. The Nakoosa plots spray solution included Sylguard (a silicone based surfactant) where as Latron was used at Habelman.

The Nakoosa plots were the only ones that were not subjected to frost protection (by spraying water) the same night, and therefore had adequate exposure to LPE. In the third group of plots (Ben Lear cultivar), early harvest (within 8 days of LPE application) further limited the benefit from LPE.

For LPE application, **each plot was divided into 4 strips**. The two outer strips were sprayed with LPE, while the two middle strips were not sprayed. Hence, each LPE sprayed strip is matched to an adjacent unsprayed strip that serves as its control. Sampling for dip and for determination of decay characteristics, sugar, acidity and color was performed as follows:

Decay: *Nine random samples (2000grams each) were taken from each strip. These were randomly divided into sets of three. Each sample was subjected to either dip in H_20 or LPE, or remained without dip according to set. (Hence resulting in three samples from each strip at each choice of dip). Three subsamples (of 400 grams each) were taken from each sample at up to three time points during storage. These three different times corresponded to:*

First evaluation: within a week after harvest

*Second evaluation: one month after storage) (**Thanksgiving Grade Out**)*

*Third evaluation: two month after storage (**Christmas Grade Out**)*

*The percentage of berries with **rot, popper, damage and disease** was determined in each sample. Additional decay outcomes were created as rot plus popper, and as sum total of all non-marketable (bad) fruit.*

***Sugar, acidity and color:** At the time of harvest **nine to eighteen samples** were taken from each strip for determination of sugar, acidity and color. In the Nakoosa plots, these samples were collected systematically within strips in such as manner that three LPE sprayed samples were matched to three adjacent control samples with the same east/west coordinate. Whereas from Habelman one sample was removed from each load (boat) as each strip was being harvested. From these samples, two subsamples were weighed (100 gram each) within a day after harvest and frozen for later quantification of color, acidity, and sugar.*

Statistical Methods:

The three groups of plots were analyzed separately. Among the three plots at Nakoosa the first one was inadequately labeled as to which berries were treated and which were control samples. Therefore, only two of the Nakoosa plots were included in the overall combined analyses of the data.

In all analyses, means, standard deviations and box plots were produced: (I) by pre-harvest treatment (LPE spray versus no spray), for the three measured parameters (sugar acidity and color) as well as (II) by post-harvest dip treatment and by the three times of evaluations.

These **descriptive analyses were followed by analysis of variance** to determine the statistical significance of any differences. Investigation of the distribution of **the fruit quality parameters** indicated that it would be appropriate to base the analysis of variance on the **square roots of the measurements to achieve normality and equal variance**. The fruit quality measurements were considered to have arisen from a **split-split plot design with replication on the subplots**. The main plots (subjected to spray or no spray with LPE) were considered arranged in blocks (essentially consisting of a treated plot with its adjacent control). The subplot treatments were the three choices of dip, and the sub-subplots arose from the three time points. **Analysis of variance was performed with PROC MIXED in SAS** specifying plots, location within plot (i.e. respective matched set of two strips), blocks (i.e. plot by locations) by treatment interaction, and replicate by dip interaction as random effects.

The **analyses of variance for sugar, acidity and color, were performed for randomized block designs with subsampling**, where blocks were the matched sets of three LPE samples and three controls within plot and location.

Finally, mean decay levels were graphed for the three groups of plots across time to display the magnitude of difference between treatment modalities.

Results for Nakoosa (Stevens) plots: Bed 2 and 3 combined analyses

A: Fruit Storage Quality Parameters

1. Pre-Harvest Spray Application

We found significantly lower levels of rot and rot+popper for LPE sprayed (pre-harvest) fruit, and borderline significantly lower levels of total damage and popper. The significant interaction effects for rot and rot+popper arouse as the lower levels of these measurements for LPE treated berries were more pronounced at the later time points.

At Thanksgiving grade out, LPE treatment reduced total bad fruit from 3.9 to 1.6%.

At Christmas grade out, LPE treatment reduced the total bad fruit from 9.1to 6.0%.

These improvements in storage quality by LPE were primarily due to reduction in fruit rot

2. Post-Harvest Dip Treatment

LPE dipped berries displayed less rot + popper during storage. The significant result for Dip for rot+popper arouse because LPE dipped berries did significantly better than H_2O dipped berries and borderline better than berries that were not dipped at all.

A post-harvest dip with LPE of untreated (control) fruit reduced the total bad fruit from 3.9 to 3.1% and from 9.1 to 8.1% at Thanksgiving and Christmas grade outs respectively.

These improvements in fruit quality by LPE were due to a reduction in rot + popper.

A post-harvest dip with LPE, of fruit that was pre-harvest treated with LPE, reduced total bad fruit from 6.0 to 5.4% and rot + popper fruit from 3.8 to 2.8% at Christmas grade out.

B: Fruit Color, Acidity and Sugar

Pre-harvest LPE application significantly increased fruit color, sugar and acidity.

LPE application increased fruit color by 9.6 % as compared to control

LPE application increased fruit sugar by 4% as compared to control

LPE application increased acidity by 5.7% as compared to control

Results for Nakoosa (Stevens) plots: Bed 1, 2 and 3 combined analyses

A: Fruit Storage Quality Parameters

We found no significant effect of pre-harvest LPE application effect on fruit storage quality parameters.

LPE dipped berries displayed significant less total bad fruit, rotted fruit, and rot + popper during storage.

A post-harvest dip with LPE of untreated (control) fruit reduced the total bad fruit from 3.5 to 2.6% and from 8.7 to 7.9% at Thanksgiving and Christmas grade outs respectively. These improvements in fruit quality by LPE were due to a reduction in rot + popper.

A post-harvest dip with LPE, of fruit that was pre-harvest treated with LPE, reduced total bad fruit from 7.9 to 6.9% and rot + popper fruit from 5.0 to 4.0% at Christmas grade out.

B: Fruit Color, Acidity and Sugar

We found that pre-harvest LPE application significantly increased fruit color, sugar and acidity.

LPE application increased fruit color by 6.2 % as compared to control

LPE application increased fruit sugar by 3.7 % as compared to control

LPE application increased acidity by 4.4 % as compared to control

Results for Habelman Stevens plots (4 Beds combined)

A: Fruit Storage Quality Parameters

There were no significant treatment or dip effects in these plots.

B: Fruit Color, Acidity and Sugar

Pre-harvest LPE application **did not increase either fruit color, sugar or acidity in these plots.**

Results for Habelman Ben Lear plots (4 Beds combined):

A: Fruit Storage Quality Parameters

Samples from these plots were taken at only two time points. Pre- or post-harvest application of LPE did not improve fruit storage quality.

B: Fruit Color, Acidity and Sugar

Pre-harvest LPE application **did** not change acidity but decreased fruit color and sugar.

Note: We found no beneficial effect of LPE on Habelman plots. We believe this is due the fact that sprinkler frost protection was employed on all the plots on the night of the LPE application. Lack of response suggests that LPE was washed away by the water sprayed with in 6-8 hours of LPE application. These results therefore suggest that LPE should not be applied when frost protection by sprinklers is needed on the following night.